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<b>(21) International Application Number:</b> PCT/US97/16196 <b>(22) International Filing Date:</b> 12 September 1997 (12.09.97)  <b>(30) Priority Data:</b> 08/726,123                      4 October 1996 (04.10.96)                      US  <b>(71) Applicant (for all designated States except US):</b> AMGEN INC. [US/US]; Amgen Center, 1840 De Havilland Drive, Thousand Oaks, CA 91320-1789 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> BREMS, David, N. [US/US]; 3778 Calle Clara Vista, Newbury Park, CA 91320 (US). TREUHETT, Michael, J. [US/US]; 824 Pamela Wood Street, Newbury Park, CA 91320 (US).  <b>(74) Agents:</b> ODRE, Steven, M. et al.; Amgen Inc., Amgen Center, 1840 De Havilland Drive, Thousand Oaks, CA 91320-1789 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> PHARMACEUTICAL COMPOSITIONS CONTAINING AN MPL LIGAND  <b>(57) Abstract</b>  The subject invention relates to compositions of mpl ligands, comprising a full-length or truncated mpl ligand having a sequence of amino acids corresponding to amino acids 7-151 through 1-332, inclusive, of native human mpl ligand, optionally covalently linked to at least one water-soluble polymer; a buffering agent selected from glutamate, phosphate, histidine, imidazole, and acetate; an excipient selected from sorbitol, sucrose, mannitol, glycerol, polyethylene glycol, and non-polar amino acids; optionally, a detergent or lipid such as Tween; optionally, an antioxidant or chelating agent selected from glutathione, methionine, citrate and EDTA; and having a pH preferably ranging from 5.0 to 6.0 (inclusive). Such compositions may be liquid (preferably, aqueous), frozen (preferably, aqueous), or lyophilized.		

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## PHARMACEUTICAL COMPOSITIONS CONTAINING AN MPL LIGAND

Field of the Invention

5           The present invention relates to compositions containing an mpl ligand, which compositions are suitable for pharmaceutical administration.

Background of the Invention

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          The native human mpl ligand is a recently cloned cytokine that appears to be the major regulator of circulating platelet levels. See Bartley, T.D. et al., *Cell* 77:1117-1124 (1994); Lok, S. et al., *Nature* 369:565-568 (1994); de Sauvage, F.J. et al., *Nature* 369:533-538 (1994); Miyazake, H. et al., *Exp. Hematol.* 22:838 (1994); and Kuter, D.J. et al., *PNAS USA*, 91:11104-11108 (1994). Native human mpl ligand, also referred to as thrombopoietin (TPO) and megapoietin, is a protein having 332 amino acids in total.

20

          Recombinant mpl ligand produced in both Chinese Hamster Ovary (CHO) and *E. coli* cells has been demonstrated to have a biological activity of specifically stimulating or increasing megakaryocytes and/or platelets *in vivo* in mice, rats and monkeys. See e.g., Hunt, P. et al., *Blood* 84(10):390A (1994). Human mpl ligands that have been truncated from the C-terminus by up to 181 amino acids retain biological activity *in vivo*. The resulting mpl ligands have sequences that correspond to amino acids 1 - 151 up to 1 - 331 of the full-length human sequence. It is also possible to remove up to the first six amino acids at the N-terminus of the human mpl ligand protein and retain biological activity. Therefore, it appears that biological activity is retained within amino acids 7 to 151 (inclusive) of the mature amino acid sequence of human mpl ligand.

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Derivatives of mpl ligands have been demonstrated to have advantageous activity to stimulate production of megakaryocytes and/or platelets in in vivo tests. See published PCT Application WO 95/26746. In particular, mpl  
5 ligands derivatized with water soluble polymers such as polyethylene glycol ("PEG") moieties are of interest in a clinical setting because they are long-lived and active in vivo.

Compositions containing mpl ligands and related  
10 derivatives have been disclosed in a general sense. See published PCT Applications WO 95/26746, WO 95/21919, WO 95/18858, and WO 95/21920. However, controlled experiments resulting in a determination of which compositions containing  
15 mpl ligands are suitably stable for pharmaceutical use, as set forth herein, have not been previously reported. Such compositions are important for practical application of mpl ligands to patients such as humans. Thus, there continues to exist a need for such compositions in the art for use in  
20 administering mpl ligands to patients so as to result in an increase in platelets.

#### Summary of the Invention

Accordingly, it is an object of this invention to provide stable compositions that are pharmaceutically  
25 acceptable, which include mpl ligands.

It is another object of this invention to provide compositions that contain mpl ligands for administration to patients.  
30

In one embodiment, the subject invention relates to compositions of mpl ligands, comprising a full-length or truncated mpl ligand having a sequence of amino acids corresponding to amino acids 7-151 through 1-332, inclusive,  
35 of native human mpl ligand, optionally covalently linked to

at least one water-soluble polymer; a buffering agent selected from glutamate, phosphate, histidine, imidazole, and acetate; an excipient selected from sorbitol, sucrose, mannitol, glycerol, polyethylene glycol, and non-polar amino acids; optionally, a detergent such as Tween; optionally, an antioxidant or chelating agent selected from glutathione, methionine, citrate and EDTA; and having a pH preferably ranging from 5.0 to 6.0 (inclusive). Such compositions may be liquid (preferably, aqueous), frozen (preferably, aqueous), or lyophilized.

Other aspects of the present invention are set forth in the detailed disclosure provided hereinbelow.

#### Brief Description of the Drawings

FIG. 1 shows the sequences of the native human cDNA for mpl ligand and the corresponding protein (SEQ ID NOS: 1 and 2). The sequences include a leader sequence (amino acids -21 through -1, inclusive) that is cleaved in vivo from the cDNA encoded protein to yield the mature protein.

#### Detailed Description of the Invention

The subject invention provides compositions including mpl ligands along with other agents that result in stable, biologically active compositions suitable for administration to subjects such as human beings.

In a first embodiment, the subject invention relates to compositions of an mpl ligand. By "mpl ligand" in its broadest sense is meant any proteinaceous molecule that has the ability to specifically bind to and activate the mpl receptor to result in the stimulation in vivo of megakaryocyte and/or platelet production. In a preferred embodiment, the mpl ligand has an amino acid sequence identical to one obtainable from a human, such as amino acids

1-332 of the native human sequence (SEQ ID NO: 2). In another preferred embodiment, the mpl ligand has an amino acid sequence identical to at least amino acids 7-151 of SEQ ID NO: 2, preferably 1-171  $\pm$  20 amino acids (i.e., amino acids 1-151 through 1-191) particularly preferably 1-161  $\pm$  10 amino acids corresponding to SEQ ID NO: 2. Some specific preferred species of mpl ligands are the following: amino acids 1-151, 1-152, 1-153, 1-154, 1-163, 1-174, 1-191, 1-232, 1-244 of SEQ ID NO: 2. The most preferred species has amino acids 1-163 of SEQ ID NO: 2.

The mpl ligands may also be derivatized with one or more water soluble polymers, such as one or more polyethylene glycol (PEG) groups. The polymer selected should be water soluble so that the mpl ligand to which it is attached does not precipitate in an aqueous environment, such as a physiological environment. Examples of water soluble polymers are set forth in published PCT Application WO 95/26746, which is hereby incorporated by reference.

The water soluble polymers may be attached using chemical reactions such as those described in published PCT Application WO 95/26746. Preferred attachment chemistries are acylation and alkylation. The mpl ligand derivatives of this invention may be attached to multiple polymeric molecules, for example, they may contain 2-6, preferably 2-5, polymer groups attached. The polymer groups are usually attached to the protein at the alpha or epsilon amino groups of amino acids, but it is also contemplated that the polymer groups could be attached to any amino group attached to the protein which is sufficiently reactive to become attached to a polymer group under suitable reaction conditions.

In a preferred embodiment, a single polymer molecule is attached to the mpl ligand. In such cases, the polymer selected to react with the mpl ligand should be modified to have a single reactive group, such as an active

ester for acylation or an aldehyde for alkylation, so that the degree of polymerization may be controlled.

The polymer may be branched or unbranched.

Preferably, for therapeutic use of the end-product

5 preparation, the polymer will be pharmaceutically acceptable.

The water soluble polymer may be selected from the group consisting of, for example, polyethylene glycol, monomethoxy-polyethylene glycol, dextran, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, a

10 polypropylene oxide/ethylene oxide co-polymer,

polyoxyethylated polyols (e.g., glycerol) and polyvinyl

alcohol. For derivatization of the mpl ligand via an

acylation reaction, the polymer(s) selected should have a single reactive ester group. For derivatization of the mpl

15 ligand via a reductive alkylation reaction, the polymer(s) selected should have a single reactive aldehyde group.

Generally, the water soluble polymer will not be selected from naturally-occurring glycosyl residues since these are usually made more conveniently by mammalian recombinant

20 expression systems. The polymer may be of any molecular weight as long as it does not substantially interfere with or abolish biological activity of the resulting mpl ligand derivative.

A particularly preferred water-soluble polymer for  
25 use herein is polyethylene glycol, abbreviated PEG. As used herein, polyethylene glycol or PEG is meant to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono-(C1-C10) alkoxy- or aryloxy-polyethylene glycol (see, U.S. Patent 5,252,714).

30 Pegylation of an mpl ligand may be carried out by any of the pegylation reactions known in the art. See, for example: *Focus on Growth Factors* 3 (2): 4-10 (1992); EP 0 154 316; EP 0 401 384; and the other publications cited herein that relate to pegylation. Preferably, the pegylation

is carried out via an acylation reaction or an alkylation reaction with a reactive polyethylene glycol molecule.

Thus, in a preferred aspect, the present invention relates to pegylated mpl ligand, wherein the PEG group(s) is  
5 (are) attached via acyl or alkyl groups. As discussed above, such products may be mono-pegylated or poly-pegylated (e.g., containing 2-6, preferably 2-5, PEG groups). The PEG groups are generally attached to the protein at the alpha or epsilon amino groups of amino acids, but it is also contemplated that  
10 the PEG groups could be attached to any amino group attached to the protein, which is sufficiently reactive to become attached to a PEG group under suitable reaction conditions.

Preferably, the PEG group is attached via a reductive alkylation procedure and has a molecular weight of  
15 from 5 to 50 kd. In the most preferred embodiment, the PEG-mpl ligand has a PEG group that has an average molecular weight of approximately 20 kd (e.g.,  $20 \text{ kd} \pm 2 \text{ kd}$ ).

A particularly preferred mpl ligand derivative is one corresponding to amino acids 1-163 of SEQ ID NO: 2  
20 attached to a single PEG group on the alpha amino group of the first amino acid, wherein the PEG is attached via a reductive alkylation reaction with a PEG aldehyde reactant. This type of mpl ligand is referred to herein by the abbreviation "PEG-rHuMGDF".

25 In a preferred embodiment, the mpl ligand is the product of the expression of an exogenous DNA sequence that has been transfected into a host cell; that is, in a preferred embodiment the mpl ligand is a "recombinant mpl ligand". Recombinant mpl ligand may be made in any cells  
30 known for this purpose, for example, CHO cells. The preferred host is bacterial, particularly preferably *E. coli* cells. Recombinant mpl ligand is advantageously produced according to the procedures described in the publications cited herein regarding cloning and expression of mpl ligand.

Although previous workers have reported on compositions involving native human mpl ligand (amino acids 1-332 of SEQ ID NO: 2), no one has previously reported extensive stability data as a function of composition ingredients, as set forth herein. Thus, although predictions of stability of mpl ligand compositions have been made before, until the present invention, compositions having desirable stability have not been clearly established, especially for truncated, derivatized mpl ligands.

Based on the data presented hereinbelow, the present inventors have discovered certain stable compositions which contain a full-length or truncated mpl ligand having a sequence of amino acids corresponding to amino acids 7-151 through 1-332, inclusive, of native human mpl ligand, optionally covalently linked to at least one water-soluble polymer; a buffering agent selected from glutamate, phosphate, histidine, imidazole, and acetate; an excipient selected from sorbitol, sucrose, mannitol, glycerol, polyethylene glycol, and non-polar amino acids; optionally, a detergent such as Tween; optionally, an antioxidant or chelating agent selected from glutathione, methionine, citrate and EDTA; and having a pH preferably ranging from 5.0 to 6.0 (inclusive). Such compositions may be liquid (preferably, aqueous), frozen (preferably, aqueous), or lyophilized.

The concentration of the protein (mpl ligand) in the final compositions should generally range from about 0.1 mg/ml to 5 mg/ml, preferably 0.2 mg/ml to 3 mg/ml, particularly preferably 0.3 to 1 mg/ml.

Preferably, the buffer will be acetate at a concentration of from 5 to 20 mM, particularly preferably, about  $10 \pm 2$  mM. A summary of specific buffers and concentrations is provided in the following Table 1:

TABLE 1

	Buffer	Preferred Conc. Range (mM)	Working Conc. Range (mM)	Exemplary Conc. (mM)
5	Acetate	5-20	8-12	10
	Phosphate	5-20	8-12	10
	<u>Histidine</u>	<u>5-20</u>	<u>8-12</u>	<u>10</u>

10

The pH of the compositions will vary depending on the particular buffer and other factors. The preferred pH range for enhanced stability with appropriate acidic buffers (e.g., acetate) is 4.0-6.0. A more preferred range is 4.5-  
15 5.5, with about 5.0 being a most preferred embodiment.

The compositions should also contain an excipient. Some exemplary excipients and representative concentrations are listed in Table 2:

20

TABLE 2

	Excipient	Preferred Conc. Range (W/V)	Working Conc. Range (W/V)	Exemplary Conc. (W/V)
25	sorbitol	3 - 10%	4 - 6%	5%
	sucrose	5 - 10%	8 - 10%	9%
	<u>mannitol</u>	<u>3 - 10%</u>	<u>4 - 6%</u>	<u>5%</u>

30

The excipients will generally be added in an amount so as to result in an isotonic solution.

The compositions may further contain an amino acid, which in some cases will enhance stability. Amino acids may  
35 be polar or non-polar, with non-polar amino acids being

preferred. Exemplary polar amino acids are arginine and lysine, and exemplary non-polar amino acids are glycine, proline, and alanine.

An antioxidant or chelating agent may also be included in the compositions of this invention. Preferred antioxidants are: EDTA, ascorbic acid, glutathione, methionine and citrate. Combinations of these agents are also contemplated, for example, citrate plus EDTA. Such agents are included in an amount suitable to reduce or eliminate oxidation of the mpl ligand. Exemplary concentrations are: 0.1 - 10 mM, preferably, 0.5 - 5 mM, typically 1 - 3 mM.

A detergent or lipid may also be included in the compositions of this invention. Some representative detergents are: Tween brand of polysorbate (e.g., Tween 20 and Tween 80); Brij 35; Pluronic (e.g., F-127 and F-68); sodium dodecyl sulfate; Triton (e.g., X-100); dimyristoyl phosphatidyl glycerol (DMPG); PEG castor oil (e.g., PEG-40); oleth-3-phosphate; diethanolamine oleth-10-phosphate; and a mixture (e.g., 1:1) of short, long chain unilamellar vesicles (SLUV) containing, e.g., C8 (caprylic) and C14 (myristic) lipids. These detergents/lipids are generally included in an amount sufficient to prevent loss of mpl ligand due to sticking to surfaces or aggregation. Some exemplary detergent concentrations are 0.004 mg/ml - 50 mg/ml; preferably, 0.004 mg/ml - 10 mg/ml; most preferably, 0.006 - 0.060 mg/ml. The need to include these detergents/lipids will be greater when the concentration of mpl ligand is lower, such as especially  $\leq 0.2$  mg/ml of mpl ligand.

Such compositions may be liquid (preferably, aqueous), frozen (preferably, aqueous), or lyophilized.

With regards to lyophilized compositions, there is the possibility of increased protein aggregation as compared to liquid compositions. A particularly preferred lyophilized composition contains a combination of glutamate, sucrose and

mannitol at a pH within the range of 4.0-6.0. A list of particularly preferred compositions is provided in the following Table 3:

5

TABLE 3

	<u>Material</u>	<u>Range</u>	<u>Example</u>
	Glutamate	5-20 mM	10 mM
	Sucrose	2-10% (w/v)	6% (w/v)
10	Mannitol	1-5% (w/v)	2% (w/v)
	<u>pH</u>	<u>4.0-6.0</u>	<u>5.0</u>

The compositions of this invention are "stable", by which is meant that they retain at least about 87 %, preferably about 90 %, most preferably about 93 %, of intact mpl ligand derivative after storage for 12 weeks at a temperature of 37°C as analyzed by SEC chromatography only (see Table 4). This degree of stability is important in a practical sense because less stability would result in unacceptable safety concerns for patients.

A "therapeutically effective amount" as used herein refers to that amount which provides a suitable biological effect in a subject, usually a therapeutic effect for a given condition and administration regimen in a patient.

The present compositions can be systemically administered parenterally, intravenously or subcutaneously. When systemically administered, the therapeutic compositions for use in this invention may be in the form of a pyrogen-free, physiologically acceptable aqueous solution. The specific route chosen will depend upon the condition being treated. The required dosage will be in amounts sufficient to raise the platelet and/or megakaryocyte levels of patients and will vary depending upon the severity of the condition being treated, the method of administration used and the like.

The conditions to be treated by the methods and compositions of the present invention are generally those which involve an existing megakaryocyte/platelet deficiency or an expected megakaryocyte/platelet deficiency in the future (e.g., because of planned surgery). Such conditions will usually be the result of a deficiency (temporary or permanent) of active mpl ligand *in vivo*. The generic term for platelet deficiency is thrombocytopenia, and hence the methods and compositions of the present invention are generally useful for treating thrombocytopenia.

Thrombocytopenia (platelet deficiencies) may be present for various reasons, including chemotherapy and other therapy with a variety of drugs, radiation therapy, surgery, accidental blood loss, and other specific disease conditions. Exemplary specific disease conditions that involve thrombocytopenia and may be treated in accordance with this invention are: aplastic anemia, idiopathic thrombocytopenia, metastatic tumors which result in thrombocytopenia, systemic lupus erythematosus, splenomegaly, Fanconi's syndrome, vitamin B12 deficiency, folic acid deficiency, May-Hegglin anomaly, Wiskott-Aldrich syndrome, and paroxysmal nocturnal hemoglobinuria. Also, certain treatments for AIDS result in thrombocytopenia (e.g., AZT). Certain wound healing disorders might also benefit from an increase in platelet numbers.

With regard to anticipated platelet deficiencies, e.g., due to future surgery, an mpl ligand analog of the present invention could be administered several days to several hours prior to the need for platelets. With regard to acute situations, e.g., accidental and massive blood loss, an mpl ligand analog could be administered along with blood or purified platelets.

Mpl ligand compositions may also be administered to normal human subjects who plan to donate platelets or other related cells in the future. Administration of a composition

of this invention would increase the amount of platelets and/or related cells that the patient could donate at one time.

The dosage regimen involved in a method for  
5 treating the above-described conditions will be determined by the attending physician, considering various factors which modify the action of drugs, e.g. the age, condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors.  
10 Generally, the daily regimen should be in the range of 0.01-1000 micrograms of mpl ligand analog per kilogram of body weight.

The compositions of the present invention may also be employed, alone or in combination with other cytokines,  
15 soluble Mpl (i.e., mpl ligand) receptor, hematopoietic factors, interleukins, growth factors or antibodies in the treatment of disease states characterized by other symptoms as well as platelet deficiencies. It is anticipated that such compositions will prove useful in treating some forms of  
20 thrombocytopenia in combination with general stimulators of hematopoiesis, such as IL-3 or GM-CSF. Other megakaryocytic stimulatory factors, i.e., meg-CSF, stem cell factor (SCF), leukemia inhibitory factor (LIF), oncostatin M (OSM), or other molecules with megakaryocyte stimulating activity may  
25 also be employed with mpl ligand. Additional exemplary cytokines or hematopoietic factors for such co-administration include IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-11, colony stimulating factor-1 (CSF-1), GM-CSF, granulocyte colony stimulating factor (G-CSF), EPO,  
30 interferon-alpha (IFN-alpha), IFN-beta, or IFN-gamma. It may further be useful to administer, either simultaneously or sequentially, an effective amount of a soluble mammalian Mpl receptor, which appears to have an effect of causing megakaryocytes to fragment into platelets once the  
35 megakaryocytes have reached mature form. Thus,

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administration of PEG-mpl ligand (to enhance the number of mature megakaryocytes) followed by administration of the soluble Mpl receptor (to inactivate the analog and allow the mature megakaryocytes to produce platelets) is expected to be a particularly effective means of stimulating platelet production. The dosage recited above would be adjusted to compensate for such additional components in the therapeutic composition. Progress of the treated patient can be monitored by conventional methods.

10

The following examples are offered to more fully illustrate the invention, but are not to be construed as limiting the scope thereof.

15

#### EXAMPLE 1

The following Tables 4 and 5 are a summary of data provided in some of the following examples. In each of these examples, the mpl ligand tested was PEG-rHuMGDF, which contains amino acids 1-163 of SEQ ID NO: 2, mono-pegylated at the alpha amino group of the N-terminal amino acid with a polyethylene glycol group having an average molecular weight of about 20 kDa.

20

TABLE 4

Formulation	Percent Main Peak as Measured by SEC <sup>1</sup> after 12 weeks at 37°C
Histidine, pH 8.0, 5% Sorbitol	70
Tris, pH 8.0, 5% Sorbitol	47
Phosphate, pH 7.0, 5% Sorbitol	71
Histidine, pH 7.0, 5% Sorbitol	84
Phosphate, pH 6.0, 5% Sorbitol	89
Glutamate/Histidine, pH 6.0, 5% Sorbitol	92
Histidine, pH 6.0, 5% Sorbitol	92
Imidazole, pH 6.0, 5% Sorbitol	92
Glutamate, pH 5.5, 5% Sorbitol	91
Glutamate/Histidine, pH 5.5, 5% Sorbitol	93
Acetate, pH 5.0, 5% Sorbitol	92
Glutamate/Histidine, pH 5.0, 5% Sorbitol	93
Glutamate, pH 5.0, 5% Sorbitol	91
Histidine, pH 5.0, 5% Sorbitol	89
Succinate, pH 5.0, 5% Sorbitol	76
Glutamate, pH 4.0, 5% Sorbitol	87
Succinate, pH 4.0, 5% Sorbitol	81
Acetate, pH 4.0, 5% Sorbitol	77
Tartrate, pH 4.0, 5% Sorbitol	18
Succinate, pH 3.5, 5% Sorbitol	74

<sup>1</sup>Additional stability indicating assays, specifically,  
5 reversed phase and cation exchange chromatography, gave  
similar results to SEC.

The percent decrease in main peak indicates a  
preferred pH range of 4.0 - 6.0, preferably 5.0 - 6.0. In  
10 addition, buffer effects within the pH range 4.0 - 6.0

indicate that certain buffers within this range are not preferred.

TABLE 5

Formulation	Percent Main Peak as Measured by SEC <sup>1</sup> after 12 weeks at 37°C
Acetate, pH 5.0, 5% isotonic polyols (Sorbitol)	92
Acetate, pH 5.0, isotonic saline, both mono- and divalent (NaCl)	10
Acetate, pH 5.0, isotonic polar amino acids (Lysine)	10
Acetate, pH 5.0, isotonic nonpolar amino acids (Glycine)	84

5

All polyols tested, which include: sorbitol, sucrose, glycerol, mannitol and polyethylene glycol, showed similar results.

10 All salts (both monovalent and divalent), which include: NaCl, CaCl<sub>2</sub>, CuCl<sub>2</sub>, MgCl<sub>2</sub>, MnCl<sub>2</sub>, NiCl<sub>2</sub>, ZnCl<sub>2</sub> and FeCl<sub>2</sub>, showed similar results.

All polar amino acids tested, which include: arginine, and lysine, showed similar results.

15 All nonpolar amino acids tested, which include: glycine, proline, and alanine, showed similar results.

All antioxidants tested which include: EDTA, ascorbic acid, glutathione, methionine, methionine + EDTA and citrate, did not show substantial increases in the stability of PEG-rHuMGDF beyond A50S (see Example 2 for definition).

20

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## EXAMPLE 2

pH Evaluation

5 A. Starting Material: PEG-rHuMGDF

B. Formulations:

	10mM Acetate pH 5.0, 5% Sorbitol	(A50S)
	10mM Acetate pH 4.0, 5% Sorbitol	(A40S)
10	10mM Succinate pH 3.5, 5% Sorbitol	(S35S)
	10mM Succinate pH 4.0, 5% Sorbitol	(S40S)
	10mM Succinate pH 5.0 5% Sorbitol	(S50S)
	10mM Histidine pH 6.0, 5% Sorbitol	(H60S)
	10mM Imidazole pH 6.0, 5% Sorbitol	(I60S)
15	10mM Tartrate pH 4.0, 5% Sorbitol	(T40S)
	10mM Glutamate pH 4.0, 5% Sorbitol	(E40S)
	10mM Phosphate pH 6.0, 5% Sorbitol	(P60S)
	10mM Phosphate pH 7.0, 5% Sorbitol	(P70S)
	10mM Tris pH 8.0 5% Sorbitol	(T80S)
20	10mM Histidine pH 5.0, 5% Sorbitol	(H50S)
	10mM Histidine pH 6.0, 5% Sorbitol	(H60S)
	10mM Histidine pH 7.0, 5% Sorbitol	(H70S)
	10mM Histidine pH 8.0, 5% Sorbitol	(H80S)

25 C. Vials: 1mL in 3cc vials filled at a protein concentration of  
0.5 mg/mL

D. Temp & Time points: 37°C; time points indicated on Tables

30 E. Analyses: HPLC: Size exclusion chromatography (SEC),  
reverse phase chromatography (RP),  
ion exchange chromatography (IEX)

## F. Data

Tables 6-11 show the percent main peak by size exclusion, reversed phase and cation exchange chromatography after incubation at 37°C for the times indicated.

**pH Evaluation - Percent Main Peak by Size Exclusion  
Chromatography After Incubation at 37°C for the Time  
Indicated**

**TABLE 6**

Form. *	Incubation Time					
	T=0	T=2 Weeks	T=4 weeks	T=8 weeks	T=12 Weeks	T=17 Weeks
A40S	94.39	90.86	88.20	82.91	77.21	70.19
S35S	94.90	93.65	90.29	80.95	74.28	62.43
S40S	94.63	92.59	90.20	86.16	80.51	72.34
S50S	94.27	87.53	85.29	80.77	76.45	71.12
H60S	94.99	94.31	93.77	91.74	89.13	85.96
I60S	94.93	94.56	94.20	92.77	91.49	94.83
T40S	89.71	43.46	34.44	24.48	18.10	-
E40S	94.90	94.29	93.65	91.30	86.84	77.94

\*Form. = Formulation

TABLE 7

5

Form. *	Incubation Time							
	T=0	T=3 Days	T=7 Days	T=10 Days	T=2 Weeks	T=3 Weeks	T=6 Weeks	T=12 Weeks
T80S	97.70	93.56	91.28	88.12	86.63	80.25	66.18	46.58
H50S	97.53	94.20	94.22	94.37	94.54	92.94	91.76	89.18
H60S	97.62	97.02	97.24	97.09	94.50	96.19	95.12	91.68
H70S	97.62	96.64	96.75	95.86	95.37	94.33	90.95	84.34
H80S	97.70	-	94.68	93.27	92.03	89.36	81.52	69.65
P60S	97.65	95.55	96.84	96.35	96.38	95.77	93.76	89.16
P70S	97.81	96.45	95.84	94.83	94.12	91.77	84.81	71.14
A50S	97.72	96.28	95.43	95.45	95.63	94.71	93.97	91.65

10

**pH Evaluation - Percent Main Peak by Reversed Phase  
Chromatography After Incubation at 37C for the Time Indicated**

15

TABLE 8

Form. *	Incubation Time					
	T=0	T=2 Weeks	T=4 weeks	T=8 weeks	T=12 Weeks	T=17 Weeks
A40S	93.49	91.69	84.34	78.87	70.55	64.23
S35S	93.78	90.10	80.86	70.36	59.35	50.98
S40S	93.91	91.61	83.89	77.16	-	59.22
S50S	93.49	92.59	88.93	85.96	79.35	73.71
H60S	93.65	90.54	86.69	86.49	80.88	76.11
I60S	93.68	92.61	87.64	87.34	84.11	79.49
T40S	93.65	88.04	74.51	66.08	53.87	-
E40S	93.67	92.39	86.03	80.64	71.50	65.23

TABLE 9

5

Form. *	Incubation Time						
	Time Zero	T=3 Days	T=7 Days	T=10 Days	T=2 Weeks	T=6 Weeks	T=12 Weeks
T80S	95.96	93.75	92.68	88.57	87.98	68.58	48.84
H50S	96.14	95.38	95.52	94.70	97.08	92.18	88.45
H60S	95.88	94.32	94.77	94.65	94.08	90.20	84.80
H70S	95.78	92.66	93.84	90.72	90.95	-	70.83
H80S	95.88	94.11	93.23	90.94	89.66	77.82	67.64
P60S	96.49	94.62	94.82	94.18	93.44	86.25	81.09
P70S	96.04	94.98	94.14	92.41	94.37	82.67	71.11
A50S	96.02	95.13	95.57	94.68	96.35	92.78	89.30

10

**pH Evaluation - Percent Main Peak by Cation Exchange  
Chromatography After Incubation at 37C for the Time Indicated**

TABLE 10

15

Form. *	Incubation Time					
	T=0	T=2 Weeks	T=4 weeks	T=8 weeks	T=12 Weeks	T=17 Weeks
A40S	81.17	73.84	69.66	53.96	43.09	41.14
S35S	82.64	72.97	67.10	52.36	45.84	37.70
S40S	82.16	74.54	70.95	53.81	44.63	34.96
S50S	82.86	75.30	67.91	58.14	49.48	41.49
H60S	82.32	79.60	75.57	70.77	65.47	-
I60S	83.72	80.20	77.95	72.15	64.05	66.01
T40S	79.23	30.25	27.58	19.37	11.92	-
E40S	82.37	75.53	69.71	59.80	50.91	47.72

TABLE 11

5

Form. *	Incubation Time							
	Time Zero	T=3 Days	T=7 Days	T=10 Days	T=2 Weeks	T=3 Weeks	T=6 Weeks	T=12 Weeks
T80S	87.44	80.38	78.04	76.41	72.76	71.28	54.49	-
H50S	84.42	83.70	83.86	83.23	82.16	78.52	76.07	70.85
H60S	87.86	86.81	85.40	84.56	82.00	78.90	80.64	73.71
H70S	82.99	83.33	81.35	81.01	79.55	72.70	67.95	56.91
H80S	84.08	84.72	81.49	82.17	77.28	64.21	68.21	54.79
P60S	85.68	84.58	83.73	83.22	79.15	75.87	73.38	60.93
P70S	87.60	78.70	82.50	84.04	78.99	77.85	78.90	69.18
A50S	83.00	84.54	81.65	83.81	81.54	77.68	79.50	72.22

## EXAMPLE 3

10

Mpl ligand Concentration Evaluation

A. Starting Material: PEG-rHuMGDF

15 B. Formulations:

10mM Acetate pH 5.0, 5% Sorbitol, 2.0 mg/mL (20A5S)

10mM Acetate pH 5.0, 5% Sorbitol, 1.0 mg/mL (10A5S)

10mM Acetate pH 5.0, 5% Sorbitol, 0.5 mg/mL (05A5S)

10mM Acetate pH 5.0, 5% Sorbitol, 0.2 mg/mL (02A5S)

20

C. Vials: 1mL in 3cc vials filled at the protein concentrations indicated

D. Temp &amp; Time points: 37°C; time points indicated on Tables

25

E. Analyses: HPLC: SEC, RP, IEX

## F. Data

Tables 12-14 show the percent main peak by size exclusion, reversed phase and cation exchange chromatography after incubation at 37°C for the times indicated.

**Mpl Ligand Concentration Evaluation - Percent  
Main Peak After Incubation at 37°C for the Time Indicated**

**TABLE 12****Size Exclusion Chromatography**

Form. *	Incubation Time						
	T=0	T=1 Week	T=2 Weeks	T=3 Weeks	T=4 Weeks	T=8 Weeks	T=12 Weeks
02A5S	94.44	94.86	93.84	94.53	94.51	93.90	92.74
05A5S	94.11	93.78	92.86	93.25	93.40	91.55	90.11
10A5S	93.96	92.60	91.69	91.74	91.32	89.20	86.89
20A5S	93.81	91.50	90.25	90.20	89.55	86.89	83.59

\*Form. = Formulation

**TABLE 13****Reversed Phase Chromatography**

Form. *	Incubation Time						
	T=0	T=1 Week	T=2 Weeks	T=3 Weeks	T=4 Weeks	T=8 Weeks	T=12 Weeks
02A5S	94.53	93.41	93.28	92.16	91.05	88.89	87.90
05A5S	94.73	94.44	93.30	92.41	91.97	89.18	85.97
10A5S	94.86	94.56	93.25	92.39	91.98	88.06	87.62
20A5S	94.67	94.12	93.07	92.47	91.79	88.37	87.07

TABLE 14

5

## Cation Exchange Chromatography

Form. *	Incubation Time					
	T=0	T=1 Week	T=2 Weeks	T=4 Weeks	T=8 Weeks	T=12 Weeks
02A5S	79.40	65.75	78.56	79.80	74.42	59.74
05A5S	78.22	67.44	80.26	81.10	76.27	61.39
10A5S	-	66.18	80.80	82.02	75.81	60.72
20A5S	79.52	76.71	82.09	81.38	74.26	60.89

10

## EXAMPLE 4

Excipient Evaluation

A. Starting Material: PEG-rHuMGDF

15

B. Formulations:

20

10mM Acetate pH 5.0, 5% Sorbitol,  
5 mM EDTA (A5SE)

10mM Acetate pH 5.0, 2% Alanine (A5A)

10mM Acetate pH 5.0, 1.6% Glycine (A5G)

10mM Acetate pH 5.0 2.7% Proline (A5P)

10mM Acetate pH 5.0, 3.5% Lysine (A5K)

10mM Acetate pH 5.0, 4.3% Arginine (A5R)

25

10mM Glutamate pH 5.0, 9.3% Sucrose (E5Su)

10mM Glutamate pH 5.0, 5% Sorbitol (E5S)

C. Vials: 1mL in 3cc vials filled at a protein concentration of  
0.5 mg/ml

30

D. Temp & Time points: 37°C; time points indicated on Tables

E. Analyses: HPLC: SEC, RP, IEX

F. Data

5

Tables 15-17 show the percent main peak by size exclusion, reversed phase and cation exchange chromatography after incubation at 37°C for the times indicated.

10

**Excipient Evaluation - Percent Main Peak After Incubation at 37C for the Time Indicated.**

15

**TABLE 15**

**Size Exclusion Chromatography**

20

Formulation	Incubation Time				
	T=0	T=2 Weeks	T=4 Weeks	T=8 Weeks	T=12 Weeks
A5R	84.71	21.83	19.01	12.30	-
E5Su	94.30	91.40	91.95	88.36	86.52
A5G	94.57	91.22	91.43	92.26	84.50
A5K	86.75	17.24	15.02	9.76	-
A5SE	93.08	60.01	54.53	43.22	37.75
A5P	94.66	91.97	92.16	87.63	85.05
E5S	94.78	93.07	93.55	90.68	92.28
A5A	94.55	91.57	92.18	88.13	85.89

TABLE 16

5

## Cation Exchange Chromatography

Formulation	Incubation Time				
	T=0	T=2 Weeks	T=4 Weeks	T=8 Weeks	T=12 Weeks
A5R	82.26	28.41	21.32	11.71	-
E5Su	84.93	86.22	88.62	72.16	65.04
A5G	86.17	81.90	66.81	18.95	26.45
A5K	80.88	20.18	14.23	8.75	-
A5SE	84.56	62.75	56.40	36.31	30.38
A5P	86.73	87.58	86.22	69.34	65.76
E5S	87.49	90.23	88.83	75.55	70.01
A5A	86.28	81.31	86.10	70.29	63.69

10

TABLE 17

## Reversed Phase Chromatography

Formulation	Incubation Time			
	T=0	T=2 Weeks	T=4 Weeks	T=12 Weeks
A5R	95.96	92.35	85.68	-
E5Su	96.10	94.57	91.59	77.92
A5G	95.71	69.61	39.86	-
A5K	95.81	88.93	81.30	-
A5SE	95.98	93.60	90.01	-
A5P	95.67	92.98	89.96	78.85
E5S	95.64	93.98	90.61	83.90
A5A	95.78	90.38	86.52	70.00

15

## EXAMPLE 5

Isotonicity Evaluation

20

A. Starting Material: PEG-rHuMGDF

## B. Formulations:

	10mM Acetate pH 5.0, 9.3% Sucrose	(A5SU)
	10mM Acetate pH 5.0, 5% Mannitol	(A5MA)
5	10mM Acetate pH 5.0, 140 mM NaCl	(A5N)
	10mM Acetate pH 5.0, 2% PEG 8000	(A5P8)
	10mM Acetate pH 5.0, 2.5% Glycerol	(A5G)
	10mM Acetate pH 5.0, 5% Sorbitol, .01% Tween 20	(A5ST)
10	10mM Histidine pH 6.0, 5% Sorbitol	(H6S)
	10mM Histidine pH 6.0, 5% Sorbitol, .001% Ascorbic Acid	(H6AA)

C. Vials: 1mL in 3cc vials filled at a protein concentration of  
15 0.5 mg/ml

D. Temp & Time points: 37°C; time points indicated on Tables

E. Analyses: HPLC: SEC, RP, IEX  
20

F. Data

Tables 18-20 show the percent main peak by size  
exclusion, reversed phase and cation exchange chromatography  
25 after incubation at 37°C for the times indicated.

30 **Isotonicity Evaluation - Percent Main Peak After Incubation at  
37°C for the Time Indicated.**

TABLE 18

5

## Size Exclusion Chromatography

Form. *	Incubation Time						
	T=0	T=3.5 Days	T=1 Week	T=2 Weeks	T=3 Weeks	T=7 Weeks	T=12 Weeks
H6S	96.70	96.23	96.30	95.95	95.77	93.37	91.03
H6Aa	96.75	95.66	96.03	95.72	95.29	93.32	90.61
A5N	83.54	30.42	28.04	24.64	20.19	17.62	-
A5Ma	96.55	95.14	95.22	95.02	95.13	93.08	91.42
A5Su	96.36	95.18	95.37	95.16	95.60	93.49	91.89
A5G	96.46	95.29	95.49	95.20	95.59	93.23	91.68
A5P8	94.88	92.89	92.89	92.61	91.62	92.78	82.05
A5ST	96.25	94.83	94.50	94.58	94.37	91.16	87.68

\*Form. = Formulation

10

TABLE 19

5

## Reversed Phase Chromatography

Form. *	Incubation Time					
	T=0	T=3.5 Days	T=1 Week	T=2 Weeks	T=7 Weeks	T=12 Weeks
H6S	95.49	94.39	94.69	85.55	87.60	85.62
H6Aa	95.64	92.00	90.67	93.02	74.22	66.22
A5N	94.94	93.99	92.19	89.52	68.67	-
A5Ma	95.90	95.05	95.29	94.34	90.13	87.22
A5Su	95.55	95.39	94.78	93.47	88.19	82.88
A5G	95.57	94.78	95.51	94.88	90.04	88.64
A5P8	94.75	91.31	87.07	76.93	13.41	-
A5ST	94.57	93.24	93.90	82.75	87.61	85.45

10

TABLE 20

15

## Cation Exchange Chromatography

Form. *	Incubation Time						
	T=0	T=3.5 Days	T=1 Week	T=2 Weeks	T=3 Weeks	T=7 Weeks	T=12 Weeks
H6S	87.13	85.79	84.67	82.66	82.77	79.32	59.35
H6Aa	85.12	80.75	79.31	75.99	75.23	64.62	45.93
A5N	79.98	39.01	29.14	25.03	20.77	14.67	-
A5Ma	84.73	84.37	84.43	81.92	83.07	78.17	58.52
A5Su	82.98	84.83	83.46	80.76	81.09	76.61	56.85
A5G	82.79	84.69	85.09	81.73	81.61	76.98	59.03
A5P8	85.65	82.26	81.74	77.41	74.77	53.74	4.80
A5ST	85.02	83.83	83.95	80.02	79.79	74.56	55.85

## EXAMPLE 6

Anti-oxidant/Chelating Agent Evaluation

5

A. Starting Material: PEG-rHuMGDF

B. Formulations:

10	10 mM Acetate pH 5.0, 5% Sorbitol	
	3 mM glutathione	(A5S GT)
	10 mM Acetate pH 5.0, 5% Sorbitol	
	5 mM methionine	(A5S M)
	10 mM Acetate pH 5.0, 5% Sorbitol	
15	5 mM methionine, 1 mM EDTA	(A5S ME)
	10 mM Acetate pH 5.0, 5% Sorbitol	
	1 mM citrate	(A5S C)
	10 mM Acetate pH 5.0, 5% Sorbitol	
	0.5 mM citrate	(A5S 05C)
20	10 mM Acetate pH 5.0, 5% Sorbitol	
	1 mM EDTA	(A5S 1E)
	10 mM Acetate pH 5.0, 5% Sorbitol	
	0.5 mM EDTA	(A5S E)

25 C. Vials: 1mL in 3cc vials filled at a protein concentration of  
0.5 mg/ml

D. Temp & Time points: 37°C; time points indicated on Tables

30 E. Analyses: HPLC: SEC, RP, IEX

## F. Data

Tables 21-23 show the percent main peak by size exclusion, reversed phase and cation exchange chromatography after incubation at 37°C for the times indicated.

Anti-Oxidant/Chelating Agent Evaluation - Percent Main Peak After Incubation at 37°C for the Time Indicated.

TABLE 21

## Size Exclusion Chromatography

Formulation	Incubation Time			
	T=0	T=2 Weeks	T=4 Weeks	T=9 Weeks
A5SGT	16.69	12.32	8.03	5.91
A5SM	94.53	91.03	89.72	89.00
A5SME	94.88	92.15	90.73	85.74
A5SC	94.30	90.83	88.14	79.61
A5S05C	93.96	90.95	88.98	-
A5S1E	94.88	90.75	89.15	80.63
A5SE	94.80	91.72	89.89	82.06

TABLE 22

## Reversed Phase Chromatography

Formulation	Incubation Time				
	T=0	T=2 Weeks	T=4 Weeks	T=9 Weeks	T=12 Weeks
A5SGT	5.69	2.37	2.80	2.60	2.50
A5SM	94.70	91.59	88.67	88.55	86.83
A5SME	94.54	87.66	91.36	81.97	80.39
A5SC	93.98	81.13	90.33	68.01	62.91
A5S05C	94.43	87.01	84.30	79.83	76.35
A5S1E	94.36	86.84	76.43	89.93	84.56
A5SE	94.75	90.27	85.64	81.20	78.96

TABLE 23

## Cation Exchange Chromatography

5

Formulation	Incubation Time			
	T=0	T=2 Weeks	T=4 Weeks	T=9 Weeks
A5SGT	4.66	5.15	0.0	-
A5SM	84.69	81.18	80.96	72.95
A5SME	84.93	78.36	78.26	69.48
A5SC	85.23	72.11	66.46	54.68
A5S05C	86.06	78.00	72.53	66.06
A5S1E	85.38	77.00	76.42	66.21
A5SE	86.51	80.07	77.93	69.79

10

## EXAMPLE 7

Detergent Evaluation

A. Starting Material: PEG-rHuMGDF

15

B. Formulations:

All forms contain 10mM acetate at pH 5.0, with  
5% sorbitol and 0.050 mg/ml PEG-rHuMGDF.

20

004T20 : 0.004 mg/ml Tween-20

006T20 : 0.006 mg/ml Tween-20

010T20 : 0.010 mg/ml Tween-20

040T20 : 0.040 mg/ml Tween-20

060T20 : 0.060 mg/ml Tween-20

25

004T80 : 0.004 mg/ml Tween-80

006T80 : 0.006 mg/ml Tween-80

010T80 : 0.010 mg/ml Tween-80

040T80 : 0.040 mg/ml Tween-80

060T80 : 0.060 mg/ml Tween-80

30

## C. Data:

Table 24 shows the results of reverse phase HPLC purity based on the percent main peak:

5

TABLE 24

FORMULATION	INCUBATION TIME AT 37 DEGREES CELSIUS				
	T=0 WEEKS	T=2 WEEKS	T=4 WEEKS	T=6 WEEKS	T=12 WEEKS
A5S	94.66	94.05	90.67	90.90	89.07
004T20	94.68	94.79	91.25	91.02	88.49
006T20	94.69	93.99	91.48	90.46	88.43
010T20	94.89	94.30	91.33	90.76	88.47
040T20	94.59	92.95	90.63	89.88	87.58
060T20	94.61	92.62	90.57	89.49	86.72
004T80	94.44	94.35	90.91	90.80	88.48
006T80	94.43	94.05	91.01	90.18	88.08
010T80	94.89	94.22	90.69	90.22	88.04
040T80	94.45	92.64	89.74	89.26	86.56
060T80	94.19	88.38	88.67	87.64	84.53

## D. Results:

Detergents such as Tween may be included in PEG-rHuMGDF formulations to enhance physical stability and recovery without detrimental effects to chemical stability. Tween-20 and Tween-80 may be added to PEG-rHuMGDF formulations up to final concentrations of about 0.060 mg/ml without causing excessive methionine oxidation. Tween-20 and Tween-80 are most effective in the concentration range of 0.006 mg/ml to 0.060 mg/ml.

## EXAMPLE 8

Lyophilized Compositions

5

The following Table 25 is a summary of data obtained for lyophilized compositions including an mpl ligand. In each of these examples, the mpl ligand tested was PEG-rHuMGDF, which contains amino acids 1-163 of SEQ ID NO: 2, mono-PEGylated at the alpha amino group of the N-terminal amino acid with a polyethylene glycol group having an average molecular weight of about 20 kDa. For Tables 25-27, the lyophilized PEG-rHuMGDF was reconstituted with about 1 ml of water for injection prior to analysis and the percent main peak represents the recovery of PEG-rHuMGDF as a consequence of lyophilization.

10

15

TABLE 25

Formulation	% main peak by SEC
10mM histidine, 5% mannitol	88
10mM histidine, 4% mannitol, 1% sucrose	93

20 Note: Mpl ligand concentration was 0.5 mg/ml.

For the lyophilized samples no additional physical stability was achieved in pH's 6, 7 and 8 as measured by size exclusion.

25

Range of sucrose concentrations studied:

TABLE 26

5

Sucrose Conc.	% main peak by SEC
2%	91
4%	94
5%	97
6%	95

Range of buffers (10mM concentration) studied:

TABLE 27

10

Formulation	% main peak by SEC HPLC
histidine, 3.8% mannitol, 2% sucrose, pH 5	87
citrate, 3.8% mannitol, 2% sucrose, pH 5	87
acetate, 3.8% mannitol, 2% sucrose, pH 5	79
succinate, 3.8% mannitol, 2% sucrose, pH 5	88
MES, 3.8% mannitol, 2% sucrose, pH 5	88
phosphate, 3.8% mannitol, 2% sucrose, pH 7	87
phosphate, 3.8% mannitol, 0.5% glycine, pH 7	80

All stabilizing agents such as amino acids (e.g. isotonic arginine, lysine, proline and histidine) and

amorphous agents (e.g., trehalose and PEG) did not show improved stability during lyophilization.

Formulation with best main peak recovery and lowest levels of aggregation:

5

10mM glutamate, 6% sucrose, 2% mannitol, pH 5.0.

10 While the invention has been described in what is considered to be its preferred embodiments, it is not to be limited to the disclosed embodiments, but on the contrary, is intended to cover various modifications and equivalents included within the spirit and scope of the appended claims, which scope is to be accorded the broadest interpretation so as to encompass all such modifications and equivalents.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: AMGEN INC.
- (ii) TITLE OF INVENTION: PHARMACEUTICAL COMPOSITIONS CONTAINING  
AN MPL LIGAND
- (iii) NUMBER OF SEQUENCES: 2
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: AMGEN INC.
  - (B) STREET: 1849 DeHavilland Drive
  - (C) CITY: Thousand Oaks
  - (D) STATE: California
  - (E) COUNTRY: US
  - (F) ZIP: 91320-1789
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: US (Not Yet Assigned)
  - (B) FILING DATE: 04-OCT-1996
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: COOK Ph.D., Robert R.
  - (B) REGISTRATION NUMBER: 31,602
  - (C) REFERENCE/DOCKET NUMBER: A-412

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1342 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: unknown
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 36..1097

## (ix) FEATURE:

(A) NAME/KEY: mat\_peptide  
(B) LOCATION: 99..1097

## (ix) FEATURE:

(A) NAME/KEY: sig\_peptide  
(B) LOCATION: 36..98

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CAGGGAGCCA CGCCAGCCAA GACACCCCGG CCAGA ATG GAG CTG ACT GAA TTG	53
Met Glu Leu Thr Glu Leu	
-21 -20	
CTC CTC GTG GTC ATG CTT CTC CTA ACT GCA AGG CTA ACG CTG TCC AGC	101
Leu Leu Val Val Met Leu Leu Leu Thr Ala Arg Leu Thr Leu Ser Ser	
-15 -10 -5 1	
CCG GCT CCT CCT GCT TGT GAC CTC CGA GTC CTC AGT AAA CTG CTT CGT	149
Pro Ala Pro Pro Ala Cys Asp Leu Arg Val Leu Ser Lys Leu Leu Arg	
5 10 15	
GAC TCC CAT GTC CTT CAC AGC AGA CTG AGC CAG TGC CCA GAG GTT CAC	197
Asp Ser His Val Leu His Ser Arg Leu Ser Gln Cys Pro Glu Val His	
20 25 30	
CCT TTG CCT ACA CCT GTC CTG CTG CCT GCT GTG GAC TTT AGC TTG GGA	245
Pro Leu Pro Thr Pro Val Leu Leu Pro Ala Val Asp Phe Ser Leu Gly	
35 40 45	
GAA TGG AAA ACC CAG ATG GAG GAG ACC AAG GCA CAG GAC ATT CTG GGA	293
Glu Trp Lys Thr Gln Met Glu Glu Thr Lys Ala Gln Asp Ile Leu Gly	
50 55 60 65	
GCA GTG ACC CTT CTG CTG GAG GGA GTG ATG GCA GCA CGG GGA CAA CTG	341
Ala Val Thr Leu Leu Leu Glu Gly Val Met Ala Ala Arg Gly Gln Leu	
70 75 80	
GGA CCC ACT TGC CTC TCA TCC CTC CTG GGG CAG CTT TCT GGA CAG GTC	389
Gly Pro Thr Cys Leu Ser Ser Leu Leu Gly Gln Leu Ser Gly Gln Val	
85 90 95	
CGT CTC CTC CTT GGG GCC CTG CAG AGC CTC CTT GGA ACC CAG CTT CCT	437
Arg Leu Leu Leu Gly Ala Leu Gln Ser Leu Leu Gly Thr Gln Leu Pro	
100 105 110	
CCA CAG GGC AGG ACC ACA GCT CAC AAG GAT CCC AAT GCC ATC TTC CTG	485
Pro Gln Gly Arg Thr Thr Ala His Lys Asp Pro Asn Ala Ile Phe Leu	
115 120 125	

AGC TTC CAA CAC CTG CTC CGA GGA AAG GTG CGT TTC CTG ATG CTT GTA	533
Ser Phe Gln His Leu Leu Arg Gly Lys Val Arg Phe Leu Met Leu Val	
130 135 140 145	
GGA GGG TCC ACC CTC TGC GTC AGG CGG GCC CCA CCC ACC ACA GCT GTC	581
Gly Gly Ser Thr Leu Cys Val Arg Arg Ala Pro Pro Thr Thr Ala Val	
150 155 160	
CCC AGC AGA ACC TCT CTA GTC CTC ACA CTG AAC GAG CTC CCA AAC AGG	629
Pro Ser Arg Thr Ser Leu Val Leu Thr Leu Asn Glu Leu Pro Asn Arg	
165 170 175	
ACT TCT GGA TTG TTG GAG ACA AAC TTC ACT GCC TCA GCC AGA ACT ACT	677
Thr Ser Gly Leu Leu Glu Thr Asn Phe Thr Ala Ser Ala Arg Thr Thr	
180 185 190	
GGC TCT GGG CTT CTG AAG TGG CAG CAG GGA TTC AGA GCC AAG ATT CCT	725
Gly Ser Gly Leu Leu Lys Trp Gln Gln Gly Phe Arg Ala Lys Ile Pro	
195 200 205	
GGT CTG CTG AAC CAA ACC TCC AGG TCC CTG GAC CAA ATC CCC GGA TAC	773
Gly Leu Leu Asn Gln Thr Ser Arg Ser Leu Asp Gln Ile Pro Gly Tyr	
210 215 220 225	
CTG AAC AGG ATA CAC GAA CTC TTG AAT GGA ACT CGT GGA CTC TTT CCT	821
Leu Asn Arg Ile His Glu Leu Leu Asn Gly Thr Arg Gly Leu Phe Pro	
230 235 240	
GGA CCC TCA CGC AGG ACC CTA GGA GCC CCG GAC ATT TCC TCA GGA ACA	869
Gly Pro Ser Arg Arg Thr Leu Gly Ala Pro Asp Ile Ser Ser Gly Thr	
245 250 255	
TCA GAC ACA GGC TCC CTG CCA CCC AAC CTC CAG CCT GGA TAT TCT CCT	917
Ser Asp Thr Gly Ser Leu Pro Pro Asn Leu Gln Pro Gly Tyr Ser Pro	
260 265 270	
TCC CCA ACC CAT CCT CCT ACT GGA CAG TAT ACG CTC TTC CCT CTT CCA	965
Ser Pro Thr His Pro Pro Thr Gly Gln Tyr Thr Leu Phe Pro Leu Pro	
275 280 285	
CCC ACC TTG CCC ACC CCT GTG GTC CAG CTC CAC CCC CTG CTT CCT GAC	1013
Pro Thr Leu Pro Thr Pro Val Val Gln Leu His Pro Leu Leu Pro Asp	
290 295 300 305	
CCT TCT GCT CCA ACG CCC ACC CCT ACC AGC CCT CTT CTA AAC ACA TCC	1061
Pro Ser Ala Pro Thr Pro Thr Pro Thr Ser Pro Leu Leu Asn Thr Ser	
310 315 320	
TAC ACC CAC TCC CAG AAT CTG TCT CAG GAA GGG TAA GGTTCACAGA	1107
Tyr Thr His Ser Gln Asn Leu Ser Gln Glu Gly *	
325 330	
CACTGCCGAC ATCAGCATTG TCTCGTGTAC AGCTCCCTTC CCTGCAGGGC GCCCCTGGGA	1167

GACAACTGGA CAAGATTTCC TACTTTCTCC TGAAACCCAA AGCCCTGGTA AAAGGGATAC 1227  
 ACAGGACTGA AAAGGGAATC ATTTTTCAC TGTACATTATA AACCTTCAGA AGCTATTTTTT 1287  
 TTAAGCTATC AGCAATACTC ATCAGAGCAG CTAGCTCTTT GGTCTATTTT CTGCA 1342

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 354 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Glu Leu Thr Glu Leu Leu Leu Val Val Met Leu Leu Leu Thr Ala  
 -21 -20 -15 -10  
 Arg Leu Thr Leu Ser Ser Pro Ala Pro Pro Ala Cys Asp Leu Arg Val  
 -5 1 5 10  
 Leu Ser Lys Leu Leu Arg Asp Ser His Val Leu His Ser Arg Leu Ser  
 15 20 25  
 Gln Cys Pro Glu Val His Pro Leu Pro Thr Pro Val Leu Leu Pro Ala  
 30 35 40  
 Val Asp Phe Ser Leu Gly Glu Trp Lys Thr Gln Met Glu Glu Thr Lys  
 45 50 55  
 Ala Gln Asp Ile Leu Gly Ala Val Thr Leu Leu Leu Glu Gly Val Met  
 60 65 70 75  
 Ala Ala Arg Gly Gln Leu Gly Pro Thr Cys Leu Ser Ser Leu Leu Gly  
 80 85 90  
 Gln Leu Ser Gly Gln Val Arg Leu Leu Leu Gly Ala Leu Gln Ser Leu  
 95 100 105  
 Leu Gly Thr Gln Leu Pro Pro Gln Gly Arg Thr Thr Ala His Lys Asp  
 110 115 120  
 Pro Asn Ala Ile Phe Leu Ser Phe Gln His Leu Leu Arg Gly Lys Val  
 125 130 135  
 Arg Phe Leu Met Leu Val Gly Gly Ser Thr Leu Cys Val Arg Arg Ala  
 140 145 150 155

Pro Pro Thr Thr Ala Val Pro Ser Arg Thr Ser Leu Val Leu Thr Leu  
160 165 170

Asn Glu Leu Pro Asn Arg Thr Ser Gly Leu Leu Glu Thr Asn Phe Thr  
175 180 185

Ala Ser Ala Arg Thr Thr Gly Ser Gly Leu Leu Lys Trp Gln Gln Gly  
190 195 200

Phe Arg Ala Lys Ile Pro Gly Leu Leu Asn Gln Thr Ser Arg Ser Leu  
205 210 215

Asp Gln Ile Pro Gly Tyr Leu Asn Arg Ile His Glu Leu Leu Asn Gly  
220 225 230 235

Thr Arg Gly Leu Phe Pro Gly Pro Ser Arg Arg Thr Leu Gly Ala Pro  
240 245 250

Asp Ile Ser Ser Gly Thr Ser Asp Thr Gly Ser Leu Pro Pro Asn Leu  
255 260 265

Gln Pro Gly Tyr Ser Pro Ser Pro Thr His Pro Pro Thr Gly Gln Tyr  
270 275 280

Thr Leu Phe Pro Leu Pro Pro Thr Leu Pro Thr Pro Val Val Gln Leu  
285 290 295

His Pro Leu Leu Pro Asp Pro Ser Ala Pro Thr Pro Thr Pro Thr Ser  
300 305 310 315

Pro Leu Leu Asn Thr Ser Tyr Thr His Ser Gln Asn Leu Ser Gln Glu  
320 325 330

Gly \*

—

WHAT IS CLAIMED IS:

1. A composition comprising an mpl ligand, a buffer selected from the group consisting of glutamate, phosphate, histidine, imidazole, and acetate; an excipient selected from the group consisting of sorbitol, sucrose, mannitol, glycerol, polyethylene glycol, and a non-polar amino acid; and having a pH ranging from 5.0 to 6.0, inclusive.
2. A composition according to Claim 1, wherein the mpl ligand comprises at least amino acids 7-151 of SEQ ID NO: 2.
3. A composition according to Claim 1, wherein the mpl ligand consists of amino acids 1-171  $\pm$  20 amino acids of SEQ ID NO: 2.
4. A composition according to Claim 1, wherein the mpl ligand consists of amino acids 1-161  $\pm$  10 amino acids of SEQ ID NO: 2.
5. A composition according to Claim 1, wherein the mpl ligand consists of amino acids 1-151 amino acids of SEQ ID NO: 2.
6. A composition according to Claim 1, wherein the mpl ligand consists of amino acids 1-163 of SEQ ID NO: 2.
7. A composition according to Claim 1, wherein the mpl ligand is attached to a water soluble polymer selected from the group consisting of polyethylene glycol, monomethoxy-polyethylene glycol, dextran, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol

homopolymers, a polypropylene oxide/ethylene oxide copolymer, polyoxyethylated polyols.

8. A composition according to Claim 7, wherein  
5 the water soluble polymer is polyethylene glycol.

9. A composition according to Claim 6, which  
comprises acetate as the buffer, sorbitol as the excipient,  
and has a pH of about 5.0, in an aqueous medium.

10

10. A composition according to Claim 1, wherein  
the nonpolar amino acid is selected from the group consisting  
of glycine, proline, and alanine

11. A composition according to Claim 1, further  
15 comprising an antioxidant.

12. A composition according to Claim 11, wherein  
the antioxidant is selected from the group consisting of  
20 EDTA, ascorbic acid, glutathione, methionine, and citrate.

13. A composition according to Claim 1, further  
comprising a detergent or a lipid.

14. A composition according to Claim 13, wherein  
the detergent is selected from the group consisting of Tween;  
Brij 35; Pluronic; sodium dodecyl sulfate; Triton;  
dimyristoyl phosphatidyl glycerol (DMPG); PEG-40 castor oil;  
oleth-3-phosphate; diethanolamine oleth-10-phosphate; and a  
30 mixture of short and long chain unilamellar vesicles (SLUV)  
containing C8 (caprylic) and C14 (myristic) lipids.

15. A composition according to Claim 1, wherein  
the composition is in an aqueous medium.

35

16. A composition according to Claim 1, wherein the composition is in a lyophilized form.

17. A composition according to Claim 1, which  
5 comprises phosphate buffer, 5% sorbitol and has a pH of about 6.0, in an aqueous medium.

18. A composition according to Claim 1, which  
10 comprises histidine buffer, and about 5% sorbitol in an aqueous medium.

19. A composition according to Claim 1, which  
15 comprises imidazole buffer, and about 5% sorbitol in an aqueous medium.

20. A composition according to Claim 1, which  
comprises glutamate buffer, and about 5% sorbitol, in an aqueous medium.

21. A composition according to Claim 1, which  
20 comprises glutamate buffer, 5% sorbitol and has a pH of about 5.0, in an aqueous medium.

22. A composition according to Claim 1, which  
25 comprises glutamate buffer, about 6% sucrose, about 2% mannitol, a pH of about 5.0 in a lyophilized form.

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1 -21	CAGGGAGCCACGCCAGCCAAAGACACCCCGGCCAGAAATGGAGCTGACTGAATTGCTCCTC MetGluLeuThrGluLeuLeuLeu	59 -14
60 -13	GTGGTCATGCTTCTCCTAACTGCAAGGCTAACGCTGTCCAGCCCCGGCTCCTCCTGCTTGT ValValMetLeuLeuLeuThrAlaArgLeuThrLeuSerSerProAlaProProAlaCys	119 7
120 8	GACCTCCGAGTCCTCAGTAAACTGCTTCGTGACTCCCATGTCCCTTCACAGCAGACTGAGC AspLeuArgValLeuSerLysLeuLeuArgAspSerHisValLeuHisSerArgLeuSer	179 27
180 28	CAGTGCCACAGAGGTTACCCCTTTGCCCTACACCTGTCCCTGCTGCCCTGTGGACTTTAGC GlnCysProGluValHisProLeuProThrProValLeuLeuProAlaValAspPheSer	239 47
240 48	TTGGGAGAAATGGAAACCCAGATGGAGGAGACCAAGGCACAGGACATTCTGGGAGCAGTG LeuGlyGluTrpLysThrGlnMetGluGluThrLysAlaGlnAspIleLeuGlyAlaVal	299 67
300 68	ACCCTTCTGCTGGAGGAGTGATGGCAGCACGGGACAACCTGGGACCCACTTGCCCTCTCA ThrLeuLeuLeuGluGlyValMetAlaAlaArgGlyGlnLeuGlyProThrCysLeuSer	359 87
360 88	TCCCTCCTGGGGCAGCTTTCTGGACAGGTCCGTCTCCTCCTTGGGGCCCTGCAGAGCCCTC SerLeuLeuGlyGlnLeuSerGlyGlnValArgLeuLeuLeuGlyAlaLeuGlnSerLeu	419 107

FIG.1A

420	CTTGGAACCCAGCTTCCTCACAGGGCAGGACCACAGCTCACAGGATCCCAATGCCATCC	479
108	LeuGlyThrGlnLeuProProGlnGlyArgThrThrAlaHisLysAspProAsnAlaIle	127
480	TTCCCTGAGCTTCCAACACCTGCTCCGAGGAAAGGTGCGTTTCCTGATGCTTGTAGGAGGG	539
128	PheLeuSerPheGlnHisLeuLeuArgGlyLysValArgPheLeuMetLeuValGlyGly	147
540	TCCACCCCTCTGCGTCAGCGGGCCCCACCCACACAGCTGTCCCCAGCAGAACCTCTCTA	599
148	SerThrLeuCysValArgAlaProProThrThrAlaValProSerArgThrSerLeu	167
600	GTCCTCACACTGAACGAGCTCCCAAACAGGACTTCTGGATTGTGGAGACAAACTTCACT	659
168	ValLeuThrLeuAsnGluLeuProAsnArgThrSerGlyLeuLeuGluThrAsnPheThr	187
660	GCCTCAGCCAGAACTACTGGCTCTGGGCTTCTGAAGTGGCAGCAGGGATTCAGAGCCCAAG	719
188	AlaSerAlaArgThrThrGlySerGlyLeuLeuLysTrpGlnGlnGlyPheArgAlaLys	207
720	ATTCCTGGTCTGCTGAACCAACCTCCAGGTCCCTGGACCAAAATCCCCGGATACCTGAAC	779
208	IleProGlyLeuLeuAsnGlnThrSerArgSerLeuAspGlnIleProGlyTyrLeuAsn	227
780	AGGATACACGAACTCTTGAATGGAACCTCGTGGACTCTTTCCTGGACCCCTCACGCAGGACC	839
228	ArgIleHisGluLeuLeuAsnGlyThrArgGlyLeuPheProGlyProSerArgArgThr	247

FIG.1B

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840	CTAGGAGCCCCGGACATTTCTCAGGAACATCAGACACAGGCTCCCTGCCACCAACCTC	899
248	LeuGlyAlaProAspIleSerSerGlyThrSerAspThrGlySerLeuProProAsnLeu	267
900	CAGCCTGGATATTCTCCTTCCCCAACCCATCCTCTACTGGACAGTATACGCTCTTCCCT	959
268	GlnProGlyTyrSerProSerProThrHisProProThrGlyGlnTyrThrLeuPhePro	287
960	CTTCCACCCACCTTGCCCCACCCCTGTGGTCCAGCTCCACCCCTGCTTCCCTGACCCCTTCT	1019
288	LeuProProThrLeuProThrProValGlnLeuHisProLeuLeuProAspProSer	307
1020	GCTCCAACGCCCCCTACCAGCCCTCTTCTAAACACATCCTACACCCACTCCCAGAAAT	1079
308	AlaProThrProThrProThrSerProLeuLeuAsnThrSerTyrThrHisSerGlnAsn	327
1080	CTGTCTCAGGAAGGGTAAGGTTCTCAGACACTGCCGACATCAGCATTTGTCTCGTGACAG	1139
328	LeuSerGlnGlyEnd	332
1140	CTCCCTTCCCTGCAGGGCGCCCTGGGAGACAACCTGGACAAGATTTCCTACTTTCCTCTG	1199
1200	AAACCCAAAGCCCTGGTAAAGGGATACACAGGACTGAAAGGGAATCATTTTCACTGT	1259
1260	ACATTATAAACCTTCAGAAGCTATTTTTTAAGCTATCAGCAATACTCATCAGAGCAGCT	1319
1320	AGCTCTTTGGTCTATTTTCTGCA	1342

FIG.1C

# INTERNATIONAL SEARCH REPORT

Inter. Appl. No.  
PCT/US 97/16196

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C07K14/52 A61K38/19

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 97 26907 A (GENENTECH INC ;THOMAS GRIFFITH R (US)) 31 July 1997 whole document, esp. page 9ff, page 40ff, experiment 3	1
P,X	WO 96 40217 A (ZYMOGENETICS INC) 19 December 1996	1,7,15
P,Y	whole document, especially claim 25 and examples	2-6, 8-14, 16-22
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

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- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

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- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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- \*Z\* document member of the same patent family

Date of the actual completion of the international search

8 January 1998

Date of mailing of the international search report

23.01.98

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Fax (+31-70) 340-3016

Authorized officer

Kronester-Frei, A

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/16196

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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P,Y	whole document, esp. page 33ff	2-5,7-22
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X	WO 96 29989 A (AMGEN INC) 3 October 1996	1-22
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Information on patent family members

International Application No

PCT/US 97/16196

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